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Effect of Long-Term Storage on the Reliability of Blood Biomarkers for Alzheimer's Disease and Neurodegeneration

Carla R. Schubert, MS[a], **Adam J. Paulsen, MS**[a], **A. Alex Pinto, MS**[a], **Natascha Merten, PhD**[b], **Karen J. Cruickshanks, PhD**[a,b]

[a]Department of Ophthalmology and Visual Sciences, School of Medicine and Public Health, University of Wisconsin-Madison, 610 Walnut Street, Madison, WI 53726, USA

[b]Department of Population Health Sciences, School of Medicine and Public Health, University of Wisconsin-Madison, 610 Walnut Street, Madison, WI 53726, USA

Abstract

Background: Stored blood samples from longitudinal cohort studies may be useful for studying biomarkers of preclinical Alzheimer's disease. This study aimed to determine the reliability of amyloid-β_{40} and amyloid-β_{42} ($A\beta_{40}$, $A\beta_{42}$) total tau (TTau) and neurofilament light (NfL) concentrations measured in blood samples stored long-term at −80° C.

Methods: $A\beta_{40}$, $A\beta_{42}$, TTau and NfL were measured in serum and plasma samples from 2 longitudinal cohort studies. Serum samples had been stored at −80° C for 5 (n=24), 14 (n=24) and 20 years (N=78) and plasma samples had been stored for 16 years (N=78). Biomarker concentrations were measured in duplicate using a single molecule array assay (Simoa; Quanterix, Billerica, MA). Replicate samples for each sample type and storage length were included.

Results: The concentrations of $A\beta_{40}$, $A\beta_{42}$, TTau and NfL were within expected ranges. Some serum TTau concentrations were below the limit of detection. The average intra-assay coefficients of variation (CV) for duplicate measures were 2-7% for all assays except for serum TTau, which were higher (CVs 13% and 17%). Mean differences in original replicate pair $A\beta_{40}$, $A\beta_{42}$ and NfL concentrations were slightly greater in samples stored for longer versus shorter time periods.

Conclusions: $A\beta_{40}$, $A\beta_{42}$, TTau and NfL can be measured in serum and plasma samples that have been stored up to 20 years at −80° C. Long-term storage may be associated with small increases in the variability of concentrations in samples stored 14 or more years.

Keywords

Alzheimer's disease; Blood biomarkers; Epidemiology; Amyloid β; Total tau; Neurofilament light; Single molecule array; Serum; Plasma

Corresponding author: Carla R. Schubert, MS, Department of Ophthalmology and Visual Sciences, 610 Walnut Street, Rm 1087 WARF, Madison, WI 53726, USA. Phone: 608-265-3722; schubert@episense.wisc.edu.

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INTRODUCTION

Longitudinal studies have shown that brain changes associated with neurodegeneration and the development of Alzheimer's disease (AD) occur years prior to the onset of clinical symptoms [1-3]. Identifying people at high risk for neurodegeneration or AD in the early pre-clinical phase, prior to the onset of declines in cognitive function and before progression of pathophysiologic changes, may provide opportunities for behavioral and lifestyle changes, or early treatment when available, to slow the onset of clinical disease. Typically, AD type pathologic changes have been identified by neuroimaging or by measuring biomarkers such as Amyloid beta and phosphorylated tau in cerebral spinal fluid. However, these methods are not practical in the general population or large cohort studies.

More recently methods for measuring the concentrations of amyloid-β_{40} and amyloid-β_{42} ($A\beta_{40}$, $A\beta_{42}$) total tau (TTau) and neurofilament light (NfL) in blood have been developed and validated using single molecule array (Simoa) immunoassay technology [4]. These ultrasensitive assays provide potential opportunities for researchers to leverage stored blood samples from existing longitudinal cohort studies to study preclinical AD and neurodegeneration. Although previous studies have investigated the effects of pre-analytical conditions on concentrations of $A\beta_{40}$, $A\beta_{42}$, TTau, and NfL in blood, most of these studies have been focused on the effects of freeze-thaw cycles or storage at −80°C for 5 years or less [5-10]. Studies of the reliability of measuring these biomarker assays in samples that have been stored long-term (i.e., more than 5 years) at −80° Celsius(C) are lacking. Additionally, the type of stored blood samples that are available in longitudinal cohort studies may vary or be limited to one type (serum or plasma only). Levels of $A\beta_{40}$, $A\beta_{42}$, and TT are reported to be lower in serum than plasma and potentially not as reliably measured in serum [5,11,12]. The objective of this study was to determine the distribution and reliability of $A\beta_{40}$, $A\beta_{42}$, TTau and NfL concentrations measured in blood samples stored long-term at −80°C and the reliability of measuring $A\beta_{40}$, $A\beta_{42}$ and TT in serum.

METHODS

Participants

Data used in this study are from pilot studies conducted in two longitudinal cohort studies of sensory and cognitive aging: The Epidemiology of Hearing Loss Study (EHLS; 1993-2020), a population-based study in Beaver Dam, WI and, the Beaver Dam Offspring Study (BOSS; 2005-2021), a study in the adult children of EHLS participants [13-16]. EHLS data were from 78 participants with serum samples available from the 5-year examination (1998-2000) and plasma samples available from the 10-(2003-2005) year examination [13,14]. The BOSS data were from 24 participants with serum samples available from the baseline (2005-2008) and 10-year (2015-2017) examinations [15,16]. Written informed consent was obtained from all participants at each phase prior to examination and approval for this research was obtained from the University of Wisconsin Health Sciences Institutional Review Board.

Blood collection, processing, and storage

Blood collection and processing protocols were similar across the studies and phases. Participants were not required to fast prior to the blood draw at any phase. Phlebotomy was performed following standard clinical procedures. For all studies and phases, blood collection tubes were filled in a uniform order, with no additive blood collection tubes obtained prior to those containing additives. Blood obtained for serum was collected in serum separator tubes containing gel at the EHLS 5-year and BOSS 10-year examinations and, in serum tubes with no additive at the BOSS baseline examination. Samples were prepared for storage following standardized protocols. Serum tubes were held at room temperature for 30-45 minutes until the blood clotted and then were centrifuged. Blood obtained for plasma was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and tubes were centrifuged within one hour of collection.

After centrifugation, serum and plasma were immediately aliquoted into screw top polypropylene cryogenic tubes with O-rings and frozen. Cryotubes were temporarily frozen at −20° C and then moved to −80° C freezers for long-term storage. All −80° C freezers were electronically alarmed for temperature deviation. There were no instances of samples thawing due to freezer malfunction.

EHLS serum samples were assayed for NfL and plasma samples were assayed for $A\beta_{40}$, $A\beta_{42}$ and TTau. Seven serum and plasma replicate samples from each phase were included and assayed for the corresponding biomarkers. BOSS serum samples from both phases were assayed for NfL, $A\beta_{40}$, $A\beta_{42}$ and TTau and ten replicate serum samples from each phase were assayed. EHLS serum samples assayed for NfL had been through one freeze-thaw cycle prior to the current study whereas all other samples included in the current study, BOSS serum and EHLS plasma, had never been previously thawed.

All assays were conducted at the CLIA-licensed Quanterix Simoa Accelerator Laboratory (Billerica, MA, USA) in 2019 (EHLS) and 2020 (BOSS). Samples were shipped to the laboratory on dry ice. Laboratory personnel were blinded to the inclusion of replicate samples. The Simoa® Neurology 3-PlexA Kit was used to assay serum and plasma samples for $A\beta_{40}$, $A\beta_{42}$ and TTau [12]. The Simoa® NF-Light™ Advantage kit was used to assay serum samples for NfL [17]. Within a cohort, assays were completed using kits from the same lot. All samples were run in duplicate. Eight calibrators (x3) and 2 controls (x2) specific to each kit lot, were assayed on each plate. Intra-assay coefficients of variation (CV) for controls ranged from 0-10% for $A\beta_{40}$, 2-16% for $A\beta_{42}$, 1-12% for total tau, and 0-16% for NfL.

Additional data

The $A\beta_{42}$ to $A\beta_{40}$ ratio ($A\beta_{42}$/ $A\beta_{40}$) was calculated by dividing the $A\beta_{42}$ concentration by the $A\beta_{40}$ concentration for each sample. The $A\beta_{42}$/ $A\beta_{40}$ ratio was multiplied by 1,000. Storage time at −80°C was determined by calculating the years between the date the blood sample was obtained and the date the sample was assayed. Participant demographic (age, sex, education) and behavioral health data (smoking history, weekly exercise) were obtained by interview at all study phases.

Statistical Analyses

Median and interquartile (IQR) range were used to describe the distribution of the data for each study and phase. Samples were assayed in duplicate and the average of the two duplicates was used as the biomarker concentration for each sample. There were five EHLS plasma samples and one EHLS serum sample that were missing one duplicate measure due to technical issues and for these samples the one available concentration was used in place of the average. Serum TTau concentrations that were below the Limit of Detection (LOD; 0.14 pg/ml) were set to a concentration of 0.07 pg/ml (LOD/2) for calculating the median and IQR but were not included in analyses evaluating the variability and repeatability of duplicate and replicate measures. Samples with values outside the upper or lower limit of quantification (ULOQ, LLOQ) were included in all analyses.

The reliability of $A\beta_{40}$, $A\beta_{42}$, TTau and NfL concentrations measured in blood samples stored long term was assessed by evaluating the within sample variability in the duplicates and the repeatability of measurements across the original-replicate sample pairs. The variability and repeatability of the $A\beta_{42}$/ $A\beta_{40}$ ratio was similarly evaluated. The intra-assay coefficients of variation (CV) were provided by the laboratory for each (duplicate) sample assay and the average CV was calculated for each biomarker by storage time. The non-parametric Wilcoxon Sign test was used to compare mean concentrations between the duplicate measures and between the original and replicate sample pairs by storage time.

RESULTS

Study participants

Participant characteristics are shown in Table 1 by study and phase. EHLS participants (N=78) were 52-84 years (mean 65.7 years, standard deviation (SD)=8.7) of age in 1998-2000 and 38 (48.7%) were women. BOSS participants (N=24) were 46-70 years (mean 53.3 years, SD= 6.4) in 2005-2008 and 12 (50%) were women.

Storage Time at −80°C

BOSS serum samples were stored at −80° C for a mean of 4.7 (standard deviation (SD)=0.5, range 3.8-5.3) and 14.3 (SD=0.8, range 12.4-15.3) years. EHLS plasma samples assayed for $A\beta_{40}$, $A\beta_{42}$, and TTau were stored at −80° C for a mean of 15.6 years (SD=0.6, range 14.5-16.5) and serum samples assayed for NfL were stored at −80° C for a mean of 20.4 years (SD=0.6, range 19.4-21.5).

Biomarkers

Median biomarker concentrations by study cohort and phase are shown in Table 1. $A\beta_{40}$, $A\beta_{42}$ and TTau concentrations in EHLS plasma samples were higher than the concentrations in the BOSS serum samples. Serum NfL concentrations were similar in range across the studies. There were three EHLS plasma samples with an $A\beta_{40}$ concentration higher than the ULOQ (476 pg/ml). There were no other EHLS serum or plasma samples with biomarker concentrations above the ULOQ or below the LLOQ or LOD. Serum samples from the younger BOSS participants had lower TTau concentrations and, 8 of the 24 samples from

the 2005-2008 phase and 9 of the 24 from the 2015-2017 phase, were below the LOD. Nine serum samples from each phase had a TTau concentration below the LLOQ.

Variability in Duplicate Concentrations by Storage Time

Individual samples were all measured in duplicate. Within sample variability, as assessed by the average CV of duplicate measures and the difference in mean concentrations of the duplicate measures, is shown by storage time in Table 2. Overall, mean differences in duplicate concentrations for serum $A\beta_{40}$, $A\beta_{42}$ and $A\beta_{42}$/ $A\beta_{40}$ ratios were small, but differences were slightly greater for samples stored for 14 years versus 5 years (mean differences $A\beta_{40}$: 4.24 pg/ml and 1.31 pg/ml, respectively; mean differences $A\beta_{42}$: 0.23 pg/ml and 0.14 pg/ml, respectively; mean differences $A\beta_{42}$/ $A\beta_{40}$ ratio: −1.9 and 0.33, respectively) and, the difference was statistically significant for $A\beta_{40}$ samples stored for 14 years. However, the average intra-assay CVs for serum $A\beta_{40}$ and $A\beta_{42}$ were very good (2-4%) for all storage times. The mean differences in $A\beta_{40}$ and $A\beta_{42}$ duplicate concentrations in plasma samples stored 16 years were statistically significant but, the intra-assay CVs were very good (3-4%), the mean differences (Aβ40:7.64 pg/ml, SD 15.4; $A\beta_{42}$: 0.20 pg/ml, SD 0.9) were relatively small in comparison to the higher $A\beta_{40}$ and $A\beta_{42}$ concentrations in the plasma samples (Aβ40:median: 307.9 pg/ml, IQR: 266.7-338.4; Aβ42 median: 14.3, IQR: 11.7-15.8), and the mean difference in the plasma $A\beta_{42}$/ $A\beta_{40}$ ratios was small and not statistically significant (mean difference in plasma $A\beta_{42}$/ $A\beta_{40}$ ratio: −0.57)

The mean differences between duplicate TTau concentrations were also small, and similar for all storage periods, but the intra-assay CVs were higher for the BOSS serum samples (13% and 17%) versus the EHLS plasma samples (7%). The low concentrations of TTau in the BOSS serum samples, including 9 at each phase with 1 or both duplicate concentrations below the LOQ, may have increased the variability. The serum TTau concentrations below the LOD were not included in the analyses however, for all these samples (n=17), there was complete agreement as both duplicates were reported to be below the LOD.

The mean difference in duplicate NfL concentrations was the same for samples stored 5 years and 20 years (mean difference: −0.08 pg/ml) but greater for samples stored 14 years (mean difference: −0.52 pg/ml). The average intra-assay CVs for NfL were 4-6%.

Repeatability by Storage Time

Repeatability, as determined by comparing concentrations of the biomarkers in the original sample to a second blinded, replicate sample, are shown in Table 3 by storage time. For serum $A\beta_{40}$ and $A\beta_{42}$, the mean differences between the original and replicate samples were greater in samples stored 14 years versus those stored for 5 years at −80° C (mean differences $A\beta_{40}$: 14.1 pg/ml and −2.2 pg/ml, respectively, mean differences $A\beta_{42}$: 1.2 pg/ml and −0.02 pg/ml, respectively). The mean differences between the original and replicate plasma Aβ40 and $A\beta_{42}$ concentrations in samples stored for 16 years were similar in magnitude to those seen in serum stored for 14 years. The 21.9 pg/ml, or 7%, mean difference between the plasma $A\beta_{40}$ original and replicate pairs is comparable to the 14.1 pg/ml, or 9%, mean difference between the serum $A\beta_{40}$ original-replicate pairs stored for 14 years.

Similar to the results for the individual $A\beta_{40}$ and $A\beta_{42}$ original-replicate pairs, there was a slightly greater difference between the mean original and replicate serum $A\beta_{42}$/ $A\beta_{40}$ ratios for samples stored 14 versus 5 years (mean differences $A\beta_{42}$/ $A\beta_{40}$ ratios: −7.2 vs 0.4, respectively). The mean difference between the original and replicate pair $A\beta_{42}$/ $A\beta_{40}$ ratios in plasma samples stored for 16 years was very small ($A\beta_{42}$/ $A\beta_{40}$ ratio mean difference: −0.6).

The mean differences in serum TTau concentrations between the original and replicate samples were similar across storage lengths (mean differences −0.11 pg/ml and −0.09 pg/ml for 5 and 14 years storage, respectively). Three serum original-replicate pairs at both 5 and 14 years of storage time, were not included in the TTau analyses because one or both of the sample pairs had concentrations below the LOD. The mean differences in original-replicate concentrations in plasma TTau in samples stored for 16 years was also similar to that seen in serum stored for 14 years.

The NfL concentration for one serum replicate sample stored for 5 years was not available due to technical issues leaving 9 original-replicate pairs. There were small differences in serum NfL concentrations between the original and replicate samples stored 5 and 14 years at −80° C with a greater mean difference present in samples stored for 20 years (mean differences 0.9 pg/ml, 0.1 pg/ml, and 2.3 pg/ml, for 5, 14, and 20 years of storage, respectively).

DISCUSSION

The results of the current study demonstrate that $A\beta_{40}$, $A\beta_{42}$, TTau and NfL can be measured in plasma and serum samples stored up to 20 years at −80° C and, in general, long-term storage appears to have only small effects on the reliability of the measured concentrations. Whereas previous studies have shown that these biomarkers can be measured in blood samples after short term storage at −80° C [5-10], the current results add to the evidence that these biomarkers can also be reliably measured in samples stored for longer periods of time. These findings add support for the use of stored blood samples from longitudinal cohort studies for research on $A\beta_{40}$, $A\beta_{42}$, TTau and NfL as potential predictors of neurodegeneration and AD.

The concentrations and IQR of $A\beta_{40}$, $A\beta_{42}$, TTau and NfL obtained in this study were within expected ranges for the respective sample types. Because the concentrations of these biomarkers within a cohort will vary based on the attributes of the specific participants in that cohort [6, 18-20] and reference ranges have not yet been established for the general population, direct comparison to values reported in other studies is not possible. Except for 3 plasma samples with $A\beta_{40}$ concentrations above the upper LOQ, all the $A\beta_{40}$, $A\beta_{42}$, and TTau concentrations in plasma samples and $A\beta_{40}$, $A\beta_{42}$, and NfL concentrations in serum samples were within the ranges of quantification. Several serum samples from the BOSS cohort had TTau concentrations below the LOQ or the LOD. This was likely the combined effect of lower TTau concentrations in serum (versus plasma) and lower levels of TTau in the younger BOSS participants. Although we cannot rule out storage effects as a cause of the serum TTau concentrations below the LOD, there were no plasma samples

with TTau concentrations below the LOD in the older EHLS cohort in samples stored for 16 years at −80° C. Therefore, particularly for studies in middle-aged and younger populations where TTau concentrations would be expected to be lower, plasma samples may be the preferred option for measuring TTau. However, if only serum samples are available, results below the LOD are still informative and provide value for classifying participants, because it is relatively higher levels of TTau that have been associated with increased risk for neurodegeneration [21].

In the current study, we were able to evaluate differences in variability and repeatability of $A\beta_{40}$, $A\beta_{42}$, and TTau concentrations in serum samples stored for 14 years compared to 5 years. The average intra-assay CVs were very good and comparable for $A\beta_{40}$ and $A\beta_{42}$ for all storage lengths but higher for serum TTau. The slightly greater mean differences between duplicate concentrations and the original versus replicate concentrations of $A\beta_{40}$, $A\beta_{42}$ and the $A\beta_{42}/A\beta_{40}$ ratio in serum samples stored longer (14 years vs 5 years), suggests there may be marginally more variability in $A\beta_{40}$ and $A\beta_{42}$ concentrations in samples stored for longer periods. Although there was greater variability overall in serum TTau measurements, as indicated by the higher average CVs for duplicate measurements, the mean difference in duplicate concentrations was only slightly greater for samples stored for 14 years as compared to those stored 5 years and, the mean difference in original-replicate concentrations were similar across storage lengths, suggesting long-term storage had minimal effects on TTau concentrations.

The variability and repeatability of $A\beta_{40}$, $A\beta_{42}$ and TTau in EHLS plasma samples stored for 16 years appear similar to those seen in the BOSS serum samples stored for 14 years, especially for $A\beta_{42}$ and TTau but, because of the different sample types and higher concentrations measured in plasma versus serum, these comparisons should be made cautiously. Although there were statistically significant mean differences in duplicate $A\beta_{40}$ and $A\beta_{42}$ concentrations in samples stored for 16 years, the differences were small in comparison to the higher concentrations of $A\beta_{40}$ and $A\beta_{42}$ measured in the plasma samples. Additionally, while in general the variability of the $A\beta_{42}/A\beta_{40}$ ratios by storage time reflected the variability seen in the individual $A\beta_{40}$ and $A\beta_{42}$ comparisons, the mean difference between the duplicate $A\beta_{42}/A\beta_{40}$ ratios in plasma stored for 16 years was quite small and not statistically significant. It is possible that in some situations using the ratio may moderate some of the variability from pre-analytical conditions as has been reported in previous studies in cerebrospinal fluid [22,23], This issue warrants further investigation in future studies of $A\beta_{40}$ and $A\beta_{42}$ concentrations in blood.

All NfL assays were done in serum, and variability and repeatability in concentrations could be compared across samples stored for 5, 14 and 20 years. Although there was a greater mean difference in the duplicate NFL concentrations stored for 14 years versus 5 years, the mean differences in duplicate concentrations were the same for samples stored 5 years and 20 years which implies the slightly greater variability at 14 years may not be related to storage time. Conversely, there was a greater mean difference in original versus replicate NfL concentrations in samples stored for 20 years as compared to those stored 5 or 14 years which does suggest long-term storage may slightly increase the variability of NfL concentrations. It should be noted that the EHLS serum samples stored for 20 years had

also been through one freeze-thaw cycle prior to the current study whereas the BOSS serum samples stored for 5 and 14 years had never been previously thawed. Studies of the effects of freeze-thaw cycles on NfL concentrations report that 1 freeze-thaw cycle should have no or minimal effects on the variability of NfL concentrations [5,6,8].

Strengths of this study include the long storage time, up to 20 years for NfL, the relatively large number of samples available to evaluate the effects of pre-analytical conditions on concentrations of $A\beta_{40}$, $A\beta_{42}$, TTau and NfL and inclusion of both serum and plasma. A limitation of the study is the lack of pre-storage $A\beta_{40}$, $A\beta_{42}$, TTau and NfL concentrations in the samples for comparison or the availability of the same sample stored at −80° C for different lengths of time which would have allowed for more precise determination of storage effects. However, these studies are not possible as the ultrasensitive Simoa technology was not available 15-20 years ago.

Conclusion

Results from the current study demonstrate that $A\beta_{40}$, $A\beta_{42}$, TTau and NfL can be reliably measured in serum and plasma samples that have been stored up to 20 years at −80° C. Plasma may be the preferred sample type for TTau when available due to the low levels in serum. Researchers may want to consider adjusting for storage time in longitudinal studies using banked historical samples as there was slightly greater variability in $A\beta_{40}$, $A\beta_{42}$, TTau and NfL concentrations in serum and plasma samples stored 14 or more years.

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Table 1.

Participant Characteristics and Biomarker Concentrations by Study and Phase

Participant Characteristics	**EHLS 1998-2000**	**EHLS 2003-2005**	**BOSS 2005-2008**	**BOSS 2015-2017**
N	78	78	24	24
Mean age, y, (SD) (Range)	65.7 (8.7) (52-84)	70.4 (8.7) (57-88)	53.3 (6.4) (46-70)	62.9 (55-80) (6.6)
Women, N (%)	38 (48.7)	38 (48.7)	12 (50)	12 (50)
Men, N (%)	40 (51.3)	40 (51.3)	12 (50)	12 (50)
Education, y, N (%)				
≤12	49 (63.6)	49 (63.6)	11 (45.8)	11 (45.8)
>12	28 (36.4)	28 (36.4)	13 (54.2)	13 (54.2)
Current Smoker, N (%)	5 (6.5)	4 (5.2)	3 (12.5)	1 (4.2)
Exercise ≥1/week, N (%)	39 (50.7)	42 (53.9)	14 (58.3)	13 (54.2)
Sample Characteristics				
Number of samples	78	78	24	24
Sample type	Serum	Plasma	Serum	Serum
Storage Time, y, Mean (Range)	20.4 (19.4-21.5)	15.6 (14.5-16.5)	14.3 (12.4-15.3)	4.7 (3.8-5.3)
Biomarkers	**Median [IQR]**	**Median [IQR]**	**Median [IQR]**	**Median [IQR]**
NfL (pg/ml)	11.1 [8.7-17.1]	NA	11.4 [7.6-14.1]	15.9 [12.3-26.6]
$A\beta_{40}$ (pg/ml)	NA	307.9 [266.7-338.4]	146.0 [93.4-176.1]	118.9 [97.7-156.6]
$A\beta_{42}$(pg/ml)	NA	14.3 [11.7-15.8]	11.3 [7.2-14.0]	9.5 [7.4-12.4]
TTau (pg/ml)	NA	1.76 [1.34-2.33]	0.35 [0.07-0.63][a]	0.34 [0.07-0.48][a]

Aβ: Amyloid beta; BOSS: Beaver Dam Offspring Study; EHLS: Epidemiology of Hearing Loss Study; IQR: Interquartile range; NfL: Neurofilament Light; SD: Standard deviation; TTau: Total Tau; y=years.

[a]BOSS participants with Total Tau below the Limit of detection (LOD) assigned value of 0.07 (N=8 for 2005-2008; N=9 for 2015-2017.

Table 2.

Variability in Duplicate Measurements by Years Stored at −80° C

	Serum	**Serum**	**Plasma**
Storage time (years)	**5**	**14**	**16**
Dates Collected	2015-2017	2005-2008	2003-2005
$A\beta_{40}$, pg/ml (N samples)	24	24	73
Difference in duplicates, Mean (SD)	1.31 (5.3)	4.24 (12.6)	7.64 (15.4)
p difference	0.54	0.02	<0.01
Average CV	2%	4%	3%
$A\beta_{42}$, pg/ml (N samples)	24	24	73
Difference in duplicates, Mean (SD)	0.14 (0.6)	0.23 (0.6)	0.20 (0.9)
p difference	0.54	0.06	0.03
Average CV	4%	3%	4%
$A\beta_{42}/A\beta_{40}$ ratio (N sample pairs)	24	24	73
Difference in duplicates, Mean (SD)[a]	0.33 (4.9)	−1.92 (6.3)	−0.57 (2.4)
p difference	0.54	0.54	0.24
T-Tau, pg/ml (N samples)	15[b]	16[b]	73
Difference in duplicates, Mean (SD)	0.01 (0.11)	0.03 (0.16)	0.03 (0.23)
p difference	1.00	0.21	0.35
Average CV	13%	17%	7%
	Serum	**Serum**	**Serum**
Storage time (years)	**5**	**14**	**20**
Dates Collected	2015-2017	2005-2008	1998-2000
NfL, pg/ml (N samples)	24	24	77
Difference in duplicates, Mean (SD)	−0.08 (1.7)	−0.52 (1.0)	−0.08 (0.8)
p difference	0.31	0.06	0.36
Average CV	6%	4%	4%

Aβ: Amyloid beta; CV: Coefficient of Variation; NfL: Neurofilament Light; SD: Standard deviation; TTau: Total Tau.

[a] $A\beta_{42}/A\beta_{40}$ ratios multiplied by 1000.

[b] Serum Tau samples below Limit of Detection not included (n=9 stored for 5 years and n=8 stored for 14 years).

Table 3.

Repeatability of Biomarker Concentrations by Years Stored at −80° C

	Serum		Plasma
Storage Time at −80° C	**5 years**	**14 years**	**16 years**
$A\beta_{40}$, pg/ml (N sample pairs)	10	10	7
Original, Mean (SD)	135.9 (42.5)	150.1 (62.6)	297.7 (112.1)
Replicate, Mean (SD)	133.7 (51.7)	164.3 (39.2)	319.6 (65.5)
Difference (replicate – original)	−2.2	14.1	21.9
p-difference	1.00	1.00	1.00
$A\beta_{42}$, pg/ml (N sample pairs)	10	10	7
Original, Mean (SD)	11.10 (10.8)	12.0 (4.8)	14.0 (4.6)
Replicate, Mean (SD)	11.07 (10.3)	13.2 (3.7)	15.3 (2.0)
Difference (replicate – original)	−0.02	1.2	1.3
p-difference	0.75	0.11	0.45
$A\beta_{42}/A\beta_{40}$ ratio (N sample pairs)	10	10	7
Original, Mean (SD)[a]	83.5 (15.4)	86.8 (30.9)	49.3 (8.3)
Replicate, Mean (SD)[a]	83.9 (11.1)	79.6 (9.9)	48.7 (6.1)
Difference (replicate – original)[a]	0.4	−7.2	−0.6
p-difference	0.75	0.75	1.00
T-Tau, pg/ml (N sample pairs)	6[b]	7[b]	7
Original, Mean (SD)	0.51 (0.30)	0.58 (0.30)	1.88 (0.85)
Replicate, Mean (SD)	0.39 (0.20)	0.49 (0.18)	1.99 (0.50)
Difference (replicate – original)	−0.11	−0.09	0.12
p-difference	0.69	0.45	1.00
		Serum	
Storage Time at −80° C	**5 years**	**14 years**	**20 years**
NfL, pg/ml (N sample pairs)	9[c]	10	7
Original, Mean (SD)	23.7 (13.0)	13.0 (6.0)	16.1 (5.3)
Replicate, Mean (SD)	24.5 (12.0)	13.1 (6.3)	18.5 (5.8)
Difference (replicate – original)	0.9	0.1	2.3
p-difference	0.51	1.00	0.13

Aβ: Amyloid beta; NfL: Neurofilament Light; SD: Standard deviation; T-Tau: Total Tau.

[a] $A\beta_{42}/A\beta_{40}$ ratios multiplied by 1000.

[b] Original-Replicate pairs with 1 or both samples below the Limit of Detection (LOD) were not included (5 years: n=4; 14 years: n=3).

[c] NfL concentration was not available for n=1 replicate sample.